

University of Groningen

## Autoantibodies to acetylcholine receptors in myasthenia gravis

Limburg, Pieter Coenraad

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1985

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Limburg, P. C. (1985). *Autoantibodies to acetylcholine receptors in myasthenia gravis*. Drukkerij van Denderen.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## SUMMARY AND FINAL CONCLUSIONS

Myasthenia Gravis is an autoimmune disease clinically characterized by fluctuating weakness of the voluntary muscles and immunologically by the presence of autoantibodies to the acetylcholine receptors in the neuro-muscular junction.

In the Introduction (chapter I) the backgrounds of "auto" immune reactions are discussed in relation to "normal" immune reactions. Some models on immunoreactivity are presented in the context of the existence of autoimmune disease, with special attention to the consequences of Jerne's idiotypic - antiidiotypic network theory for self-recognition systems. It is stated that autoantibody production itself may not be abnormal. The fact that pathological concentrations of these autoantibodies are produced implies that this autoantibody production is not well regulated in autoimmunity.

Mechanisms leading to the induction of autoantibody production are presented and it is said that both autoantigen presentation and T-cell regulation of the antibody production may be abnormal in autoimmunity. In Myasthenia Gravis (MG) the major abnormality is the presence of autoantibodies to the acetylcholine receptor (AChR) and it has been shown that these antibodies are of direct pathogenic significance. To understand the pathophysiology of MG the role of the AChR in the neuro-muscular transmission is described shortly.

A second - possibly disease specific - characteristic of MG is the frequent incidence of thymic abnormalities. Many patients with MG have either a thymoma or a hyperplastic thymus with germinal centre formation. The aim of the present study was a) to study the clinical significance of the anti-AChR autoantibodies and b) to study the role of the thymus in this autoreactivity.

Chapter II describes a study in 250 patients with MG in whom the presence of anti-AChR is analysed in relation to several - clinical - parameters. It was found that there was no direct relation between the clinical severity and the anti-AChR level, when single measurements in patients were analysed. However, high anti-AChR levels were found predominantly in patients with early onset of disease and in patients with a thymoma, while low anti-AChR levels were found in patients with late onset of disease. The

absence of anti-AChR in 15% of the studied patients was related to ocular disease without generalization and to immunosuppressive therapy and/or thymectomy.

Since most of the untreated patients with generalized disease had detectable anti-AChR (95% of the patients), and "false" positive anti-AChR levels were only found in a few thymoma patients without MG and in rheumatoid arthritis patients treated with d-penicillamine - either with symptoms of MG or not (results not shown) -, the presence of anti-AChR is of great diagnostic significance.

Chapter III describes a follow-up study in 75 patients in whom the clinical state - graded on a 6 point scale - and the serum anti-AChR level were assessed frequently. The relation between changes in these two parameters was analysed in treated and untreated patients, receiving only anticholinesterase drugs. A strong correlation between these two parameters was found during corticosteroid and azathioprine therapy and in the period after thymectomy, while it was not seen in the untreated patients. Specifically no changes in anti-AChR were seen when clinical improvement was due to the effect of anticholinesterase therapy or if deterioration was caused by infection or emotion. The results after plasma-exchange were variable: the anti-AChR levels usually fell after the plasma-exchange to 50-25% of the pre-treatment levels, but the clinical effect could be very impressive, minimal or absent. Generally it was concluded that serial measurement of anti-AChR reflects the basic trend of the MG in severely affected patients.

In chapter IV the previous follow-up study is extended in 39 patients after thym(om)ectomy. In the 30 non-thymoma patients the pre-thymectomy anti-AChR levels were positively correlated with the severity of disease. Most (27/30) non-thymoma patients improved during the two-year follow-up study after thymectomy and in general a highly significant correlation was found between the change in anti-AChR and the change in clinical condition. The clinical improvement was not correlated with the histological picture of the thymus or with the preoperative anti-AChR level.

In the 9 thymoma patients the results were less consistent and most patients had to be treated with steroids or azathioprine, interfering with the effect of thymomectomy.

Chapter V shows the results of a study on in vitro IgG and

IgM product patients. Th investigate the au ter VI). Two immunoglobul of predomin tures. Seco synthesis w This result unstimulated pared to cor these abnorm may reflect thesis in MG Chapter VI p autoantibody from MG pati in patients When patient were studied AChR was muc in patients a thymoma we duction in t thymectomy. anti-AChR p serum anti-A resulting in pearance of and that th circulating In chapter V of the hype study highly to this prep of AChR and Numerous ACh thymic medul the thymic leucocyte me bearing cell cells and it duce antiidi bodies.

diated patients was  
realization and to  
my.

with generalized  
of the patients),  
e only found in a  
rheumatoid arthri-  
ne - either with  
) -, the presence  
ificance.

in 75 patients in  
point scale - and  
d frequently. The  
rameters was ana-  
s, receiving only  
relation between  
corticosteroid and  
after thymectomy,  
patients. Specifi-  
een when clinical  
anticholinesterase  
d by infection or  
nge were variable:  
the plasma-exchan-  
, but the clinical  
l or absent. Gene-  
asurement of anti-  
MG in severely af-

udy is extended in  
the 30 non-thymoma  
levels were positi-  
e. Most (27/30)  
e two-year follow-  
a highly signifi-  
change in anti-AChR  
The clinical impro-  
ological picture of  
i-AChR level.

ere less consistent  
ch steroids or aza-  
of thymectomy.

on in vitro IgG and

IgM production by peripheral blood lymphocytes from MG patients. This study was undertaken to be able to investigate the autoantibody production in these cultures (chapter VI). Two major disturbances were found in the in vitro immunoglobulin production. First, an increased production of predominantly IgG was found in the unstimulated cultures. Second, a decreased response of especially IgM synthesis was found upon Pokeweed mitogen stimulation. This resulted in a decreased ratio of IgM/IgG in both unstimulated and stimulated cultures of MG patients compared to controls. No specific influence of thymectomy on these abnormalities could be identified and these defects may reflect a general disturbance in immunoglobulin synthesis in MG.

Chapter VI presents the pokeweed mitogen induced in vitro autoantibody production by peripheral blood lymphocytes from MG patients. This in vitro production was only found in patients with relatively high serum anti-AChR levels. When patients with comparable high serum anti-AChR levels were studied, it was found that the production of anti-AChR was much higher in non-thymectomized patients, than in patients after thymectomy. The results in patients with a thymoma were intermediate. The in vitro anti-AChR production in thymectomized patients reflected the effect of thymectomy. In the non-thymectomized patients the in vitro anti-AChR production was related to the corresponding serum anti-AChR levels. It is concluded that thymectomy resulting in clinical improvement also leads to the disappearance of autoreactive B-cells from the peripheral blood and that the thymus may be the central source of these circulating autoreactive cells.

In chapter VII the results of an immuno-histological study of the hyperplastic thymus in MG are presented. In this study highly purified human AChR and monoclonal antibodies to this preparation were used to demonstrate the presence of AChR and of anti-AChR producing cells in the thymus.

Numerous AChR bearing cells could be demonstrated in the thymic medulla and in a minority of the patients also in the thymic follicles. The medullary cells did not have leucocyte membrane markers (OKT10, OKT11 and B1). The AChR bearing cells in the follicles were demonstrated to be B-cells and it is discussed that these cells probably produce antiidiotypic antibodies to the anti-AChR autoantibodies.

Finally it was demonstrated directly that autoantigen binding cells could be found in the thymic follicles. These cells proved to be large B-cells and thus represent the autoantibody producing cells. This study demonstrates a direct link between the autoantibody production in MG and the presence of germinal centres in the thymus.

#### Final conclusions

In this thesis it is shown that the autoantibodies to the acetylcholine receptor play a major role in Myasthenia Gravis. The presence of anti-AChR is of great diagnostic importance and quantitative follow-up studies are of help in monitoring the course of the disease.

Especially after thymectomy the specific effect of this treatment on the disease was reflected in the autoantibody levels. Also the in vitro autoantibody production study points to the central role of the thymus in the autoimmune response in MG. The immuno-histological study showed that autoantigen reactive cells are present in the hyperplastic thymus and these cells are most likely generated in the germinal centres, which would explain the clinical and immunological effects of thymectomy. Thymectomy results in elimination of the generation site of autoreactive B-cells, resulting in a disappearance of these cells from the circulation and finally in a decline in autoantibody production in the bonemarrow.

Sometimes thymectomy does not have a beneficial clinical effect and in these patients we did not see a decline in serum anti-AChR levels. Also, in these patients autoreactive B-cells were still present in the peripheral blood. We have to assume that in these patients either the surgical extirpation was not successful or that other production sites of autoreactive B-cells are present. These sites may be localized in ectopic thymic tissue or e.g. in peripheral lymphnodes.

In this respect patients with a thymoma have to be seen as a separate population. We have the idea that thymomectomy does not induce clinical improvement, disappearance of autoreactive B-cells from the peripheral blood and decline in serum anti-AChR. Besides, patients with a thymoma also have other autoantibodies and germinal centre formation is uncommon in the tumourous thymus. The autoimmune response in these patients - usually not HLA-B8/Dr3 - must have a

different a  
mus plays a  
and the clin  
mectomy in  
understood.  
the thymus p  
Germinal cen  
also be seen  
diseases lik  
arthritis. Y  
quent in MG  
two cell po  
reacted with  
first cells,  
dant in the  
determined wh  
to do with au  
On the other  
the germinal  
cific monoclo  
these cells  
duced an anti  
the autostimu  
to produce an  
idiotype - a  
patients and  
may be produc  
Introduction,  
be responsibl  
study it also  
globulins may  
since the num  
the follicles  
others that t  
anti-AChR/ $\mu$ g I  
may implicate  
restricted tha

Future research  
autoreactive B  
after successf  
cal effects of  
findings in t  
series of pat

antigen bin-  
les. These  
present the  
nstrates a  
in MG and

dies to the  
Myasthenia  
diagnostic  
are of help

ect of this  
autoantibody  
ction study  
e autoimmune  
showed that  
hyperplastic  
ated in the  
clinical and  
y results in  
reactive B-  
cells from  
autoantibody

ial clinical  
a decline in  
ts autoreac-  
heral blood.  
r the surgi-  
ther produc-  
esent. These  
e or e.g. in

to be seen as  
thymectomy  
pppearance of  
d and decline  
thymoma also  
formation is  
mune response  
must have a

different aetiology. Our studies made clear that the thymus plays a central role in the autoimmune reaction in MG and the clinical as well as immunological effects of thymectomy in patients with a hyperplastic thymus may be understood. However, we have not been able to explain why the thymus plays this role.

Germinal centre formation is not specific for MG, but can also be seen in other diseases, especially in autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. Yet, the germinal centres are much more frequent in MG and if present, more abundant. In our study two cell populations were found in the thymus which reacted with AChR-specific monoclonal antibodies. The first cells, reacting with Mab AChR-5-1, were very abundant in the thymic medulla in MG and it has yet to be determined whether these non-lymphoid cells have anything to do with autoantigen presentation.

On the other hand, in two patients, cells were found in the germinal centres which reacted with another AChR specific monoclonal antibody. These were B-cells and either these cells carried an AChR-like structure or they produced an antiidiotype. These cells may be responsible for the autostimulation as well. If these cells can be shown to produce antiidiotype, then it can be concluded that the idiotypic - antiidiotype network is functioning in these patients and that both the idiotypic and the antiidiotype may be produced in the same follicles. As stated in the Introduction, aberrant function of this network system may be responsible for the autoantibody production. In our study it also seemed likely that other - auto? - immunoglobulins may be produced in the thymic follicles as well, since the number of AChR binding B-cells varied widely in the follicles. This is in agreement with the finding of others that the specific anti-AChR concentration in fmol anti-AChR/ $\mu$ g IgG varies in different thymic cultures. This may implicate that the autoimmune disorder in MG is less restricted than we now suppose.

Future research will have to prove the hypothesis that autoreactive B-cells disappear from the peripheral blood after successful thymectomy. The clinical and immunological effects of thymectomy should be correlated with the findings in the immuno-histological studies in a larger series of patients. In these studies other - already

available - monoclonal antibodies could be used to confirm the present findings.

These studies could be extended to patients with a thymoma to see whether autoantigen presentation is present in the thymoma. It is expected that this mechanism will differ from patients with a hyperplastic thymus and that no auto-reactive B-cell containing germinal centres will be found. Finally, the monoclonal antibodies may be of help in isolating the AChR bearing and anti-AChR producing cells from the thymus to study the mechanisms involved in the auto-reactivity.

#### SAMENVATTING

Dit proefschrift beschrijft de rol van autoantigenpresentatie bij de ziekte die bekend staat als Myasthenia Gravis. Het gaat om een ziekte die gekenmerkt wordt door een zwakte van de spieren, die voortvloeit uit de aanwezigheid van auto-antistoffen tegen de AChR (acetylcholinreceptor). Het afweersysteem (immuunsysteem) produceert deze antistoffen, die de AChR blokkeren. Dit proces wordt gereguleerd door de presentatie van autoantigenen aan T-cellen. Het onderzoek richt zich op het begrijpen van de mechanismen die betrokken zijn bij de auto-reactiviteit van het immuunsysteem. Het wordt verwacht dat deze kennis kan helpen bij het ontwikkelen van nieuwe behandelingen voor Myasthenia Gravis. Het onderzoek wordt uitgevoerd op patiënten met Myasthenia Gravis en op cellen uit de thymus. Het doel is om te zien of autoantigenpresentatie aanwezig is in de thymus van deze patiënten en of dit kan helpen bij het isoleren van AChR- dragende en anti-AChR- producerende cellen. Dit kan helpen bij het begrijpen van de mechanismen die betrokken zijn bij de auto-reactiviteit van het immuunsysteem.